**Iranian Journal of Veterinary Science and Technology**
**Vol. 3, No. 2, 2011, 17-24**

**IJVST**

# Haemato-biochemical response to lumbar epidural anaesthesia using bupivacaine alone and in combination with certain analgesics in buffalo calves

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Received: August 9, 2011 Accepted: January 17, 2012

## Abstract

The present study was undertaken to evaluate the efficacy of bupivacaine alone and in combination with fentanyl and medetomidine for lumbar epidural analgesia in fifteen buffalo calves. Haemato-biochemical parameters viz- Hb PCV, TLC, DLC, serum glucose, Serum Total protein, Serum urea nitrogen, Serum Creatinine, AST and ALT were measured after lumbar epidural administration of bupivacaine alone (group A), bupivacaine with fentanyl (group B) and bupivacaine with medetomidine (group C) animals (@ 0.15mg/kg body wt., 0.15mg/kg body wt + 2μgm/kg body wt, 0.15mg/kg body wt.+ 15μgm/kg body wt) respectively. Haematological studies revealed a not really significant decrease in Hb, PCV and TLC in group A and B, a significant ($p$<0.05) decrease in group C animals. Differential leukocytes count showed a significant decrease ($p$<0.01) in lymphocytes and a corresponding increase in neutrophil count in all the groups. Among biochemical parameters serum glucose showed a significant ($p$<0.01) increase. There was a significant increase in ALT activity, ($p$<0.05) in all 3 groups and ($p$<0.01) in group C. While AST activity increased significantly ($p$<0.05) in group C only, the BUN and serum creatinine values increased in group C. However, the values were compensated and returned towards preadministration level by 24 hrs.

**Keywords:** Epidural anaesthesia, Bupivacaine, Fentanyl, Medetomidine, Haemato-biochemical

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## Introduction

Bupivacaine HCl (1-N-butyl-DL-piperidine-2-carboxylic acid-2, 6-dimethyl anilide hydrochloride) is a long acting anilide local anaesthetic agent being used as 0.5% solution to produce good surgical analgesia (Ekblom and Widman, 1966). Bupivacaine is metabolized by demethylation of piperidine ring and coupling to glucuronic acid in the liver and excreted through the gall bladder and kidney. The analgesic potency produced by bupivacaine has been reported as ten times more potent than procaine and six times that of lignocaine (Jenkner, 1977). Medetomidine, {(4-[2, 3] dimethyl phenyl) ethyl}-1H-imidazole), is a α-2 adrenergic agonist which is used in animals to induce sedation, analgesia and muscle relaxation. In comparison to other α-2 agonists, medetomidine is more lipophilic, more selective, more potent and also eliminated faster (Scheinin and Mc Donald, 1989). Fentanyl is a 4–acylanilino–piperidine compound which is 250 times as potent analgesic as morphine and is used to produce complete surgical anaesthesia in dogs (Lumb and Jones, 1996). Fentanyl is more fat-soluble than morphine, which contributes to its rapid onset and shorter duration of action. It is a full opioid agonist and is active at μ, κ and δ receptors. The present study was conducted to evaluate the haemato-biochemical effects of lumbar epidural anaesthesia using bupivacaine alone and in combination with medetomidine and fentanyl to create a safer and more effective epidural anaesthesia of longer duration in buffalo calves.

## Materials and Methods

Fifteen clinically healthy nondescript, male buffalo calves aging between 6 to 8 months and weighing between 55 to 75 kg were used in this study. All the animals were dewormed with albendazole @ 7.5mg /kg body weight orally one month before the time of experiment. The animals were stall-fed, clean drinking water was made available, and uniform managerial conditions were maintained for all the animals throughout the period of study. The animals were kept off-fed for 24 hours and water was withheld for 12 hours prior to the start of the experiment. The volume of the drug injected was 7 ml in all the groups after reconstituting with distilled water. The animals were divided into 3 treatment groups A, B and C comprising 5 animals in each group.

Group A- Effect of bupivacaine alone, after lumbar epidural anaesthesia

Five animals were given Bupivacaine (Astrazeneca Pharma India Ltd., Bangalore, India) alone @ 0.15 mg/kg body weight.

Group B- Effect of bupivacaine with fentanyl after lumbar epidural anaesthesia

Five animals were given Bupivacaine with Fentanyl (SUN Pharmaceutical Ltd., Gujrat, India) @ 0.15 mg/kg body weight. + 2μgm/kg body weight.

Group C- Effect of bupivacaine with medetomidine after lumbar epidural anaesthesia

Five animals were given Bupivacaine with Medetomodine (Orion Corporation FARMOS, Finland) @ 0.15 mg/kg body weight. + 15 μgm/kg body weight.

*Haematological parameters:*

Hb, PCV, TLC and DLC after the collection of blood samples (5ml) in clean dry vials containing EDTA (ethylene diaminine tetra acetic acid) before and at 30, 60, 120, 240 minute and 24 hours after injection of drug(s) and estimated by Automated Haematology blood cell Counter, SUSSI, France.

*Biochemical parameters:*

Serum was separated for estimation of Serum glucose, Serum Total protein, Serum urea nitrogen, Serum Creatinine, AST, and ALT activity after the collection of blood samples (8-10 ml) in clean dry test tubes before and at 30, 60, 120, 240 minute and 24 hours after injection of drug(s) and estimated by Logotech (India) Pvy. Ltd. Techno 168 Roma-italia.

*Statistical Analysis*

Mean, standard deviation, standard error and coefficient of variation were calculated using the standard statistical formulae. The data collected by using different combinations of anaesthetic

drugs in different group of animals were analyzed using Complete Randomized Design (CRD). The data thus obtained was analyzed by using Complete Randomized Design (CRD) using SPSS 16.0 software.

## Results

*Haematological parameters*

The Mean ± SE values of the Hb, PCV, TLC and DLC have been shown in the table 1. The Hb level showed a decreasing trend from 30 to 60 min, which became significant ($p$<0.05) at 120 min in animals of group A and group B. While in animals of group C a significant ($p$<0.05) decrease in Hb was observed from 30 min which persisted up to 120 min. These values however, returned to near pre-administration level by 24 hrs in all the three groups of animals. The PCV level showed a significant ($p$<0.05) decrease at 60 min interval in group A, and group B animals. However, in animals of group C a significant ($p$<0.05) decrease in PCV was observed between 30 to 120 min interval. The TLC level showed a not really significant decrease at 30 min and 120 min interval in group A and group B animals respectively. However, in animals of group C a significant ($p$<0.05) decrease in TLC was observed between 60 to 120 min interval. The animals of group A and B showed a significant ($p$<0.05) increase in neutrophils count at 30 min which became highly significant ($p$<0.01) at 60 min. whereas, in group C animals neutrophils count increased significantly ($p$<0.01) between 30 to 120 min interval. The animals of group A and B showed a significant ($p$<0.05) decrease in lymphocytes count at 30 min interval whereas, the decrease was highly significant ($p$<0.01) at 60 min interval. The lymphocytes count of group C animals decreased significantly ($p$<0.01) between 30 to 60 min interval. Thereafter, the lymphocytes count increased gradually and returned to resting values within 24 hrs. The animals of all the groups showed a not really significant decrease in monocytes count from 30 to120 min interval. The animals of all the groups showed a not really significant increase in eosinophils count between 30 to 60 min. However, the values returned to resting values within 24 hrs.

*Biochemical Parameters*

The Mean ± SE values of various biochemical parameters have been shown in the table 2. Blood glucose level was increased in all the groups after epidural administration of drugs. In animals of group A and B there was a significant ($p$<0.01) increase in blood glucose level between 60 to 120 min interval. However, in group C, a significant ($p$<0.01) increase in blood glucose level was observed between 30 to 120 min. Thereafter, it was decreased and returned to the normal condition by 24 hrs. The increase in blood glucose level at 30 min interval was more prominent in group C animals as compared to group A and B animals.

In animals of group A and B a significant ($p$<0.05) increase in ALT was observed between 60 to120 min after the injection. The animals of group C showed a significant ($p$<0.01) increase in ALT between 60 to 120 min interval. In animals of group A and B a not really significant increase in AST was recorded between 30 to 120 min after the injection, later on the values decreased and returned near to the base value by 24 hrs. The animals of group C, showed a significant ($p$<0.05) increase in AST between 60 to 120 min interval.

In animals of group A and B a not really significant increase in plasma creatinine was observed from 30 to 240 min after the injection. The animals of group C, showed a significant ($p$<0.05) increase in plasma creatinine between 30 to 120 min after the injection. In animals of group A and B a not really significant increase in serum urea nitrogen was observed between 30 to 120 min. In group C animals also showed a significant increase ($p$<0.05) between 30 to 120 min interval. Then it was decreased gradually and finally returned to the normal condition by 24 hrs.

Total proteins showed a not really significant decrease between 30 to 120 min in group A and B animals. In group C animals also showed a significant ($p$<0.05) decrease between 30 to 120 min. Then it increased gradually to return near to the base value by 24 hrs.

**Table 1. Effect on Haematological changes in different groups at various time intervals**

| Parameters | Groups | Time interval (min) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 120 | 240 | 24 hrs |
| Haemoglobin (gm%) | A | 9.64±0.39 | 9.54±0.40 | 9.48±0.41 | 9.40±0.41* | 9.55±0.42 | 9.61±0.39 |
| | B | 8.20±0.23 | 8.02±0.22 | 7.79±0.20 | 7.67±0.20* | 7.98±0.24 | 8.15±0.18 |
| | C | 8.58±0.30 | 8.11±0.29* | 7.76±0.27* | 7.73±0.26* | 8.14±0.28 | 8.45±0.29 |
| Packed cell volume (%) | A | 28.81±1.11 | 27.56±1.07 | 26.48±1.08* | 27.96±1.08 | 28.20±1.11 | 28.65±1.12 |
| | B | 27.67±1.32 | 26.27±1.38 | 24.47±1.38* | 25.51±1.38 | 26.16±1.38 | 26.98±1.33 |
| | C | 27.10±0.85 | 25.18±0.81* | 24.33±0.83* | 24.09±0.82* | 26.08±0.81 | 26.82±0.85 |
| Total Leucocytes Count (1000/cu.m) | A | 8.81±0.51 | 7.86±0.45 | 8.06±0.47 | 8.12±0.46 | 8.19±0.46 | 8.75±0.51 |
| | B | 6.54±0.44 | 5.96±0.32 | 5.83±0.33 | 5.76±0.33 | 5.90±0.33 | 6.45±0.45 |
| | C | 5.23±0.30 | 4.90±0.29 | 4.48±0.29* | 4.46±0.29* | 4.79±0.26 | 5.08±0.29 |
| Neutrophils (%) | A | 32.14±0.30 | 34.06±0.34* | 34.64±0.43** | 34.86±0.32* | 33.62±0.28 | 32.42±0.30 |
| | B | 32.16±0.27 | 33.86±0.19* | 35.71±0.13** | 34.34±0.19* | 33.28±0.18 | 32.56±0.26 |
| | C | 32.48±0.23 | 34.51±0.14** | 36.88±0.19** | 34.44±0.15** | 33.43±0.25* | 32.61±0.22 |
| Lymphocytes (%) | A | 58.66±0.63 | 56.70±0.71* | 54.86±0.72** | 56.85±0.53 | 57.35±0.58 | 58.58±0.63 |
| | B | 57.84±0.51 | 57.20±0.49* | 55.71±0.55** | 57.24±0.47 | 57.66±0.43 | 58.01±0.28 |
| | C | 58.62±0.34 | 57.08±0.24** | 54.63±0.31** | 57.61±0.24 | 58.59±0.13 | 58.91±0.24 |
| Monocytes (%) | A | 5.78±0.21 | 5.14±0.29 | 4.64±0.30 | 4.64±0.34 | 5.18±0.36 | 5.50±0.36 |
| | B | 5.90±0.20 | 4.72±0.17 | 4.26±0.22 | 4.14±0.17 | 4.74±0.11 | 5.21±0.32 |
| | C | 5.74±0.32 | 5.16±0.20 | 4.94±0.29 | 4.50±0.25 | 4.68±0.20 | 5.26±0.13 |
| Eiosinophils (%) | A | 3.42±0.39 | 4.10±0.37 | 4.15±0.37 | 3.65±0.32 | 3.85±0.31 | 3.50±0.35 |
| | B | 4.10±0.34 | 4.22±0.33 | 4.32±0.24 | 4.28±0.16 | 4.32±0.18 | 4.22±0.12 |
| | C | 3.16±0.36 | 3.25±0.20 | 3.55±0.24 | 3.45±0.18 | 3.30±0.15 | 3.22±0.18 |

Means bearing different superscripts differ significantly at corresponding intervals ($p<0.05$); * Significantly different from the base value within group; ** Significantly different from the base value within group ($p<0.01$).

**Table 2. Effect on Serum Biochemical Changes in different groups at various time intervals**

| Parameters | Groups | Time interval (min) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 120 | 240 | 24 hrs |
| Serum Glucose (mg/dl) | A | 60.22 ±1.51 | 62.35±1.10 | 66.60±1.54** | 66.87±1.62** | 62.75±1.11 | 60.73±1.03 |
| | B | 58.50 ± 3.10 | 60.05± 3.44 | 64.59±2.34** | 64.95 ± 2.61** | 62.88±2.62* | 59.50± 3.09 |
| | C | 57.60±1.89 | 62.12±1.98** | 65.93±1.98** | 66.38±1.98** | 59.86± 2.02 | 58.61±1.89 |
| Serum Alanine Aminotransfe-rase (U/L) | A | 22.57±0.47 | 24.23±0.56 | 25.20±0.51* | 26.66±0.35* | 24.75±0.47 | 23.25±0.37 |
| | B | 23.34± 0.46 | 24.97 ±0.48 | 26.15±0.49* | 26.94±0.48* | 25.10±0.47 | 24.14±0.46 |
| | C | 23.24±0.71 | 24.84±0.65 | 26.03±0.64** | 26.10±0.64** | 25.23±0.64* | 24.15 ±0.71 |
| Serum Aspartate AminoTransf-erase (U/L) | A | 68.43±0.52 | 68.82±0.49 | 69.16±0.60 | 69.26±0.54 | 69.02±0.56 | 68.65±0.52 |
| | B | 71.10±0.58 | 71.43±0.58 | 71.94±0.69 | 72.06±0.69 | 71.89±0.68 | 71.34±0.59 |
| | C | 70.63±0.44 | 71.82±0.44 | 72.12±0.45* | 72.26±0.44* | 71.78±0.44 | 70.85±0.43 |
| Serum Creatinine (mg/dl) | A | 1.32±0.04 | 1.38±0.04 | 1.41±0.04 | 1.51±0.04 | 1.55±0.04 | 1.54±0.04 |
| | B | 1.38±0.09 | 1.42±0.11 | 1.48±0.12 | 1.55±0.11 | 1.56±0.09 | 1.44±0.09 |
| | C | 1.34±0.10 | 1.58±0.10* | 1.68±0.10* | 1.84±0.10* | 1.86±0.10 | 1.65±0.09 |
| Serum Urea Nitrogen (mg/dl) | A | 17.64±0.41 | 19.01±0.52 | 20.76±0.56 | 22.89±0.48 | 21.79±0.44 | 18.22±0.49 |
| | B | 25.11±0.85 | 25.79±0.68 | 26.00±0.67 | 25.87±0.67 | 25.56±0.74 | 25.15±0.84 |
| | C | 18.59±1.32 | 20.09±1.20* | 21.82±1.17* | 23.76±1.09* | 22.41±1.05 | 19.00±1.10 |
| Serum Total Protein (gm/dl) | A | 7.35±0.36 | 7.30±0.31 | 7.26±0.30 | 7.24±0.35 | 7.20±0.37 | 7.37±0.33 |
| | B | 8.02±0.17 | 7.97±0.17 | 7.86±0.28 | 7.69±0.24 | 7.66±0.20 | 8.00±0.19 |
| | C | 6.99±0.25 | 6.91±0.22* | 6.83±0.21* | 6.72±0.24* | 6.75±0.26 | 6.94±0.27 |

Means bearing different superscripts differ significantly at corresponding intervals ($p<0.05$); *significantly different from the base value within group; **significantly different from the base value within group ($p<0.01$)

The data thus obtained was analyzed using Complete Randomized Design (CRD) using SPSS 16.0 software.

## Discussion

Decrease in Hb and PCV might be due to pooling of the circulating blood cells in the spleen or other reservoirs secondary to decreased sympathetic activity as also reported by Soliman *et al*. (1965) after administration of tranquilizers in dog. The decrease in Hb and PCV during the period of anaesthesia or sedation might also be due to shifting of fluids from extravascular compartment to intravascular compartment in order to maintain normal cardiac output in the animals after epidural administration of medetomidine in buffalo calves (Pratap *et al*., 2001). The decrease in TLC might be due to stress and release of ACTH on account of drug administration (Tiwari *et al*., 1996). Similar findings were also reported after epidural administration of medetomidine in buffalo calves (Pratap *et al*., 2001). There was a corresponding lymphocytopaenia in response to neutrophilia in all the groups of animal after various treatments. A rise in neutrophils count and decrease in lymphocytes count might be attributed to the adrenocortical stimulation and subsequent effect of glucocorticoids (Soliman *et al.,* 1965) on circulating neutrophils. However, Chacko, (2003) observed a decrease in neutrophils and increase in lymphocytes after epidural use of fentanyl citrate in dogs.

The serum glucose concentration depends on a wide variety of factors, and the concentration at any time is the net result of the rate of entry and removal of glucose into the circulation (Kaneko, 1989). Hyperglycaemia might be due to increased concentration of adreno-cortical hormone in blood or increased sympathetic activity and suppression of microsomal enzymes (Thurmon *et al*., 1978) or increased glucose production in the liver in buffaloes. Hsu and Hummel, (1981) reported that the α-2 agonist induced hyperglycemia and hypoinsulinaemia might be mediated by α-adrenergic receptors possibly in beta-cells of pancreatic islets which inhibit the release of insulin. The present finding corroborates the observations of Tiwari *et al*. (1999) in buffalo calves. The changes in ALT activity might be due to alteration in the cell membrane permeability in response to haemodynamic changes by these anaesthetic agents which may permit these enzymes to leak from the cells with intact membrane (Koichev *et al*., 1988). The increased permeability of ALT through plasma membrane of hepatic cells, in anaesthetized animals might have occurred due to oxidative transformation of these drugs in the liver during the process of elimination leading to increased level of activity of these enzymes in the present study (Kaneko, 1989). Similar findings were also observed by Tiwari *et al*. (1999) in buffalo calves. Alteration in serum AST and ALT activities is the immediate response to cardiac insufficiency (Lehinger, 1990). The AST is distributed widely in many tissues, but the liver, myocardium and skeletal muscles are rich in this enzyme. When there is stress or any damage to the cells of these tissues, the enzyme escapes into the blood and so the AST enzymatic activity increases in blood. This might be due to the hypoxia produced because of respiratory centre depression in group C due to systemic absorption of α -2 agonists. Some alternations might also take place in cell membrane permeability which may permit these enzymes to leak from the cells with intact membrane. It corroborates the findings of Koichev *et al*. (1988). The transient increase in serum creatinine might be attributed to the temporary inhibitory effect of these drugs on the renal blood flow and consequent decrease in glomerular filtration rate which in turn might have caused a rise in serum creatinine values. Parenteral administration of xylazine has also been reported to cause a rise in creatinine level in buffaloes (Mottelib and El. Gindhi, 1975). Similar changes in serum creatinine were also observed by Dilip Kumar, (1993) in goats after systemic administration of $\alpha_2$ agonists. The increase in serum urea nitrogen might also be attributed to the temporary inhibitory effect of drugs on renal blood flow which in turn might have caused a rise in serum urea nitrogen (Kinjavdekar, 1998).

The decrease in total proteins might be due to the increased levels of glucocorticoids, increased adrenal activity and increased protein turnover resulting in decreased plasma protein and albumin. Decrease in insulin levels might modify the general metabolism and impair protein synthesis (Schumann, 1990).

## Conclusion

On the basis of this study, it was concluded that bupivacaine alone and in combination with fentanyl and medetomidine can be safely used for lumbar epidural anaesthesia in buffalo calves. However, the changes recorded during the observation period were transient, well tolerated by the animals and soon returned to their pre-administration level. The combination of bupivacaine and medetomidine was proved to be better as it produced analgesia with quicker onset and of longer duration. Combination of bupivacaine with medetomidine can be used safely in clinical cases for surgery of thoraco-abdominal region.

## Acknowledgement

The authors are highly thankful to the Dean, College of Veterinary Science and Animal Husbandry, Anjora, Durg, Chhattisgarh (India) for providing the neccessary facilities to carry out the present study.

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Iranian Journal of Veterinary Science and Technology
Vol. 3, No. 2, 2011, 17-24

IJVST

# تغییرات هماتو بیوشیمیایی ناشی از بی حسی اپیدورال کمری با استفاده از بوپیواکائین به تنهایی و در ترکیب با برخی ضد دردهای خاص در گوساله بوفالو

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دریافت مقاله :۱۳۹۰/۵/۱۸ پذیرش نهایی: ۱۳۹۰/۱۰/۲۷

## چکیده

مطالعه حاضر به منظور بررسی اثرات تجویز بوپیواکائین به صورت تنهایی و نیز در ترکیب با فنتانیل و مدتومیدین برای ایجاد بـی دردی اپیدورال کمری در پانزده گوساله بوفالو انجام گرفته است. پارامترهـای هماتوبیوشـیمیایی نظیـر هموگلـوبین PCV، TLC، DLC، گلـوکز، پروتئین تام، نیتروژن اوره، کراتینین،AST و ALT سرم بدنبال تجویز اپیدورال کمری از بوپیواکائین به تنهـایی (گـروه A)، بوپیواکـائین بـا فنتانیل (گروه B) و بوپیواکائین با مدتومیدین (گروه C) ( با دوزهای به ترتیب ۰/۱۵میلی گرم به ازای هر کیلوگرم وزن بدن، ۰/۱۵میلی گرم + ۲ میکروگرم به ازای هر کیلوگرم وزن بدن و ۰/۱۵میلی گرم + ۰/۱۵ میکروگرم به ازای هر کیلوگرم وزن بدن) مـورد انـدازه گیـری قـرار گرفت. مطالعات خونی حاکی از کاهش غیر معنی دار هموگلـوبین، PCV و TLC در گـروه A و B و کـاهش معنـی دار در گـروه C بـود. شمارش تفریقی گلبولهای سفید بیانگر کاهش معنی دار ($p$ >0.01) لنفوسیت ها و در نتیجه افزایش در تعداد نوتروفیل ها در همه گروه بود. در میان پارامترهای بیوشیمیایی قند خون افزایش معنی داری پیدا کرده بود ($p$ <0.01). افزایش معنی داری ($p$ <0.05) در فعالیت ALT در هر ۳ گروه و ($p$ <0.01) در گروه C مشاهده شد. در حـالی کـه تنهـا در گـروه C فعالیـت AST، مقـدار BUN و کـراتینین سـرم افـزایش معنی‌داری ($p$ <0.05) نشان داد. با این حال، مقادیر به دست آمده در عرض ۲۴ ساعت به سطح پایه ای پیش از تجویز دارو رسید.

**واژگان کلیدی:** بی حسی اپیدورال کمری، بوپیواکائین، با فنتانیل، مدتومیدین، هماتوبیوشیمیایی